



ParaFishControl

New tools for diagnosis of *Enterospora nucleophila*

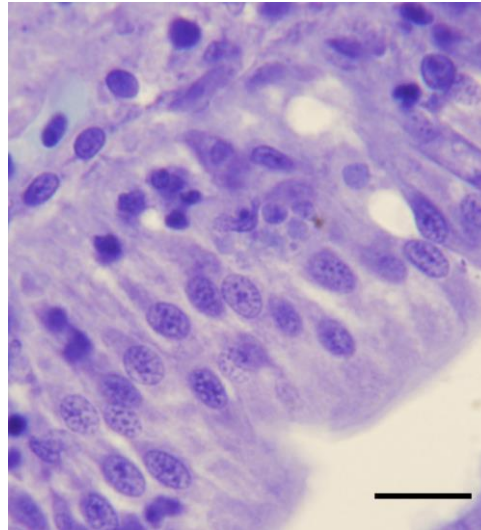
**ParaFishControl Workshop “Mediterranean Fish Parasite
Management Strategies”**

Porto, 10th September 2019

Oswaldo Palenzuela, IATS-CSIC

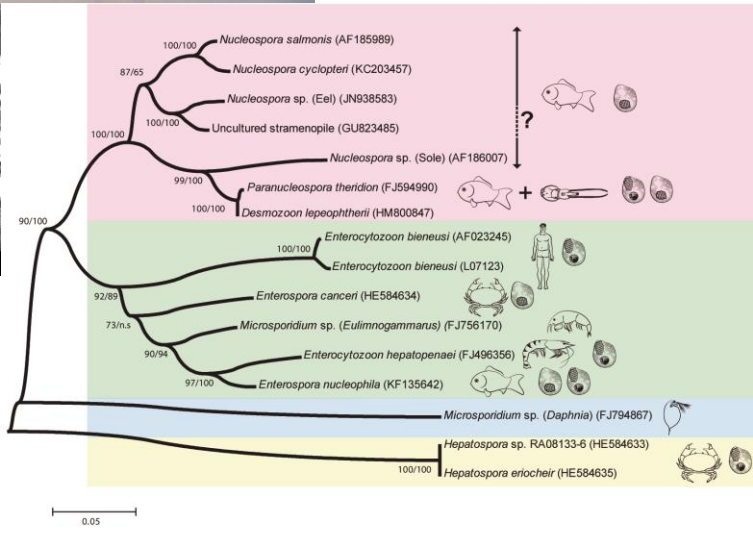
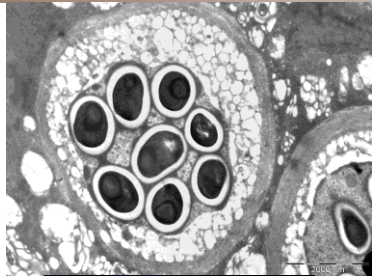


Enterospora nucleophila in GSB

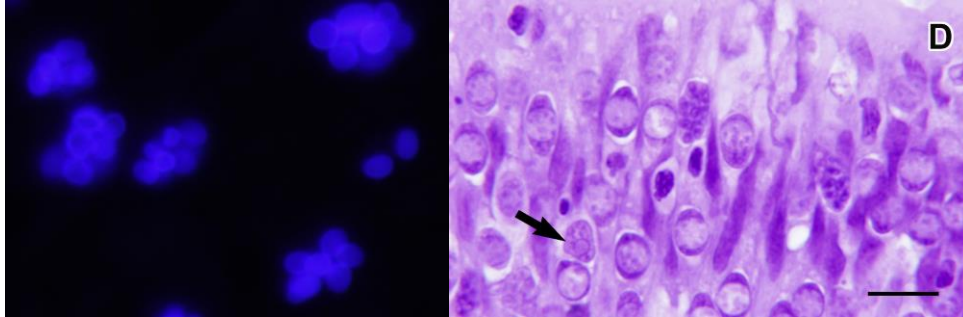


➤ Intestinal microsporidiosis, firstly detected in isolated cases in Y2000 and associated to clinical infections in Mediterranean cages in 2007

➤ Identified as Enterocytozoonidae and described in Palenzuela et al., 2014



Challenge and Impact

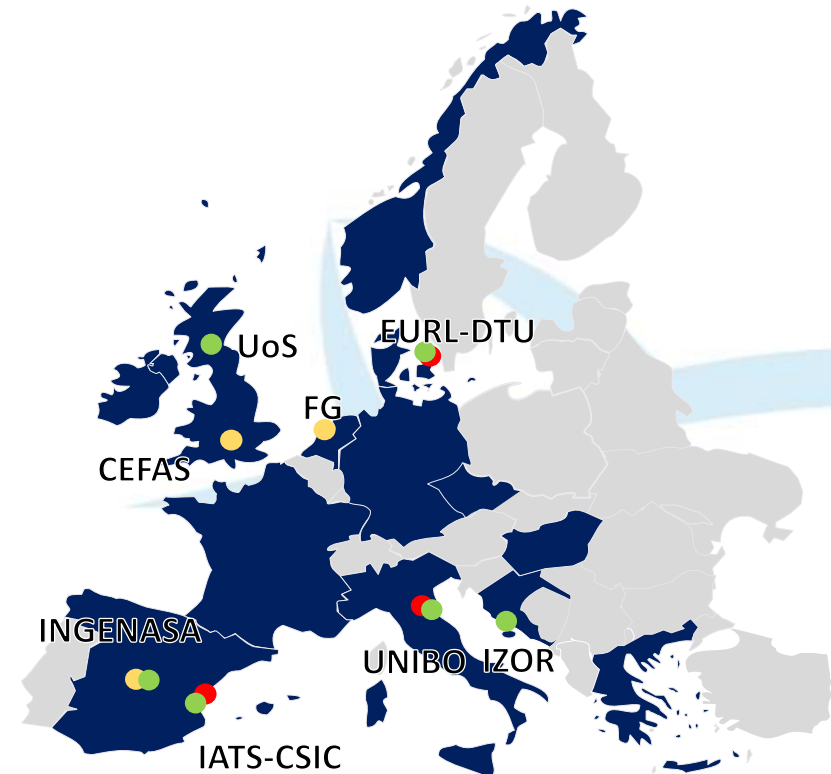
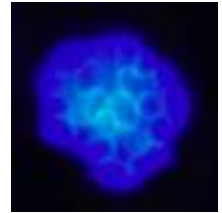


- Emerging disease with uncertain pathogeny and epidemiology
- Lack of biological background. Unknown transmission and development. No in-vivo or in-vitro models.
- Diagnostic difficulties: no pathognomonic signs, small size, intracellular, inconspicuous.

- Emaciative disease causing mortality, arrested growth and size segregation.
- Affecting mostly younger fish in winter-spring, but persistent chronic severe emaciation.
- Unknown impact for the industry, potentially high

Our approach and our team

- **Brief description of the proposed solution**
 - Develop and validate molecular diagnostic tools (WP4)
 - Reference methods and interlaboratory ringtest (WP4)
 - Epidemiological survey (longitudinal study from hatchery to harvest) (WP6)
 - Risk assessment (WP6)
 - Life cycle, in vivo and in vitro models of transmission (WP1)
 - Transcriptome/Genome data and diagnostic targets (WP1 – WP4)
 - Point-of-care tests (LFD) (WP4)
- **Team involved in the development**



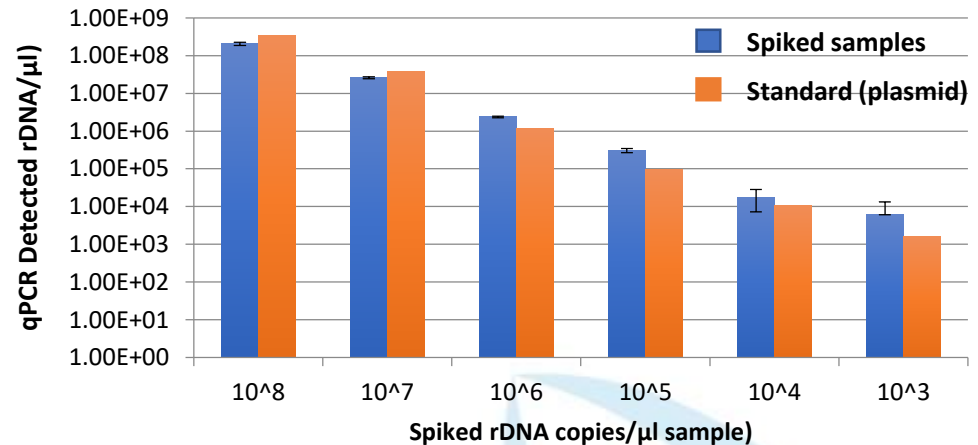
Our proposed solution (I)

qPCR test for *E. nucleophila*

Test Design and Analytical validation:

- rDNA target, qPCR optimized for detection of $1 - 10^8$ plasmid copies.
- Sensitivity $\sim 1,000$ rDNA copies in 1g intestine
- Sensitivity $\sim 10-100$ rDNA copies in 20ul blood
- Sensitivity with purified spores lower than expected (low rDNA copy number).
- Inconsistent correlation of Cts with clinical signs and spore presence.
- Patchy distribution along the intestine.

E. Nucleophila rDNA copies in 1g GSB intestine (EtOH fixed)



Interlaboratory proficiency ringtest finished
(data analysis underway)

Our proposed solution (II)

ISH Methods

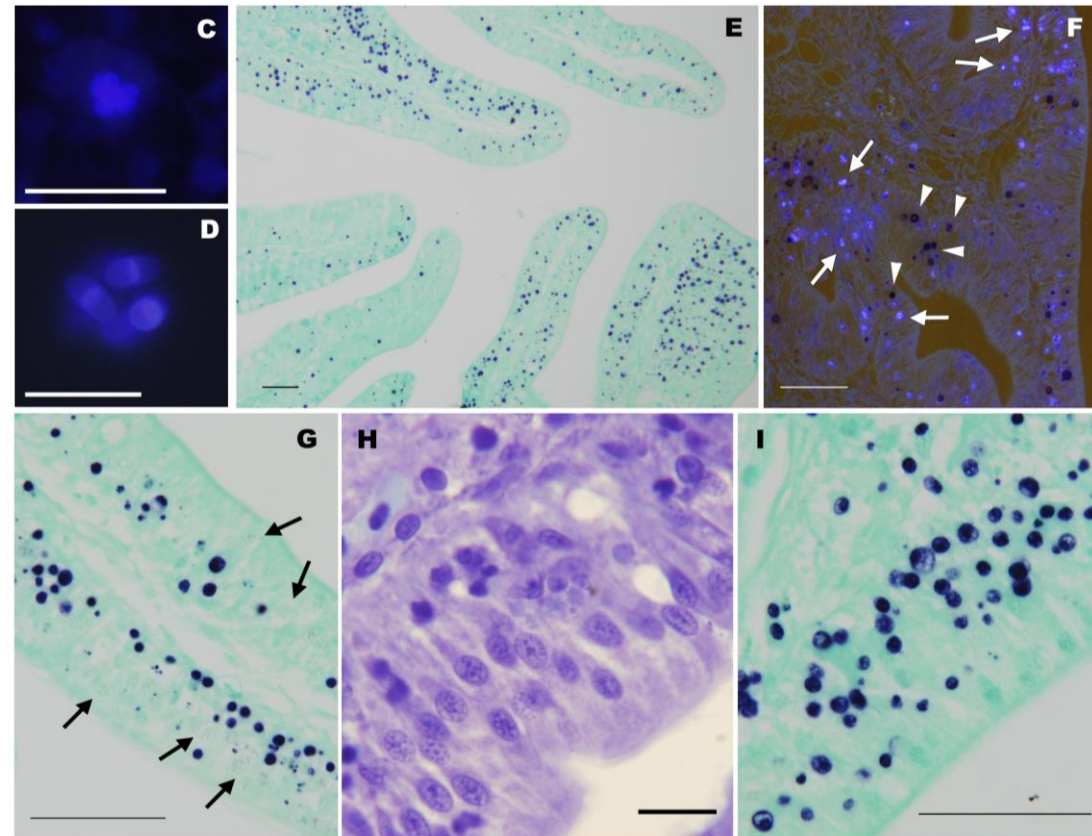
Insights in parasite development and pathogeny

Labelling of proliferative stages

Scarce labelling of spores

Better correlation with qPCR than spore detection..

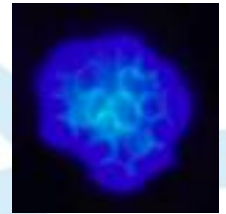
No active parasites parasites outside the intestine: only isolated spores (blood & systemic).



Hossameldin Ahmed et al. (2019) J fish Dis DOI: 10.1111/jfd.12993

Our proposed solution (III)

- Longitudinal study across Mediterranean hatcheries and cage on-growing sites
- Not found in eggs, live feed, or fry up to weaning.
- First positive cases detected during/just after nursery stage
- Variable prevalence at point of stocking into sea cages
- Present in all sea cages within 4 months of introduction
- Higher infection levels associated with worse lot performance. Up to -41% SGR in a stock with 100% prevalence at seeding.
- PCR more effective for diagnosis than histology.



Expected benefits for the industry



- Availability of reliable diagnostic methods & standard application protocols.
- Insights in parasite life cycle and disease transmission, pathogeny and impact.
- Epidemiology, economic impact and risk assessment
- Faster advances in parasite management strategies

Current status and next steps

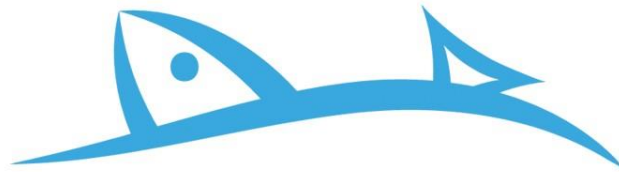
- Reference diagnostic methods developed. Interlaboratory proficiency ringtests data under analysis.
- Longitudinal study and impact pending samples at harvest.
- In vivo transmission achieved but no clinical disease produced.
- In vitro infection verified but very inefficient.
- No success with genome data. Diagnostic targets for quick diagnostic tests not identified. Still important objective.
- Multiplex tests for intestinal parasites: *Enteromyxum*, *Enterospora*, *Cryptosporidium*, others → Performfish

Conclusions

- We're just getting started with *E. nucleophila*....



Thank You



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